High prevalence of resistance to fusidic acid in clinical isolates of Staphylococcus epidermidis

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Objectives: To determine the prevalence and mechanisms of resistance to fusidic acid in clinical isolates of Staphylococcus epidermidis.

Methods: MICs of fusidic acid were determined for S. epidermidis isolates collected from the Leeds General Infirmary and from around Europe. Fusidic acid-resistant isolates were probed for the presence of the horizontally acquired resistance determinants fusB and fusC by a novel multiplex PCR assay. Mutations in the gene encoding the drug target (fusA) were detected by PCR and DNA sequencing. Resistant isolates were subjected to typing using the repeat region of the aap gene.

Results: Of 50 S. epidermidis isolates screened, 23 (46%) exhibited resistance to fusidic acid. The most common resistance determinant was fusB, found in 18 of the 23 isolates. Of the remaining isolates, two harboured fusC and three carried an identical mutation in fusA, leading to the substitution L461K in the target protein, elongation factor G. Molecular typing showed that this collection of isolates was genetically diverse.

Conclusions: This study suggests a high prevalence of resistance to fusidic acid in clinical isolates of S. epidermidis. As in Staphylococcus aureus, resistance to fusidic acid in S. epidermidis is commonly associated with the fusB determinant.

Keywords: coagulase-negative staphylococci, fusA, fusB, fusC

Introduction

The antibiotic fusidic acid is used primarily as a topical agent for treating superficial skin infections caused by staphylococci, such as atopic dermatitis and impetigo. Fusidic acid inhibits bacterial protein synthesis by binding to elongation factor G (EF-G) and preventing its release from the ribosome.1

Resistance to fusidic acid in Staphylococcus aureus and other staphylococci usually results either from mutations in fusA, the gene encoding EF-G,2 or following horizontal acquisition of the fusB or fusC determinants.2 The fusB gene encodes an EF-G-binding protein that protects the staphylococcal translation apparatus from inhibition by fusidic acid.3 An equivalent mechanism of resistance for fusC has been inferred on the basis of homology between the fusB and fusC gene products.2

Staphylococcus epidermidis is a common constituent of the commensal microflora of human skin. However, it also causes opportunistic infections and is considered a major nosocomial pathogen.4 Among the coagulase-negative staphylococci (CoNS), S. epidermidis represents one of the organisms most commonly associated with superficial skin infections in humans.5 Furthermore, this organism is thought to act as a reservoir for antibiotic resistance determinants for the major staphylococcal pathogen, S. aureus.6

To date, the prevalence and genetic basis of resistance to fusidic acid in S. epidermidis have not been examined in detail. This is of interest for several reasons. First, topical application of fusidic acid to the human skin will almost certainly produce a substantial selection pressure on S. epidermidis, whether this organism is the primary cause of an infection or simply present on the skin as a commensal. Consequently, a high prevalence of resistance to fusidic acid in this organism is expected. Secondly, if the determinants for resistance to fusidic acid in S. epidermidis are the same as those found in S. aureus, the former may act as a source of resistance for the latter, as occurs for resistance to other topical antibiotics (e.g. mupirocin).7

Here, we report on the prevalence of resistance to fusidic acid in clinical isolates of S. epidermidis and show that resistance is indeed common. Furthermore, we describe the development of a novel multiplex PCR assay for the detection of...
acquired fusidic acid resistance determinants, which was employed to establish the genetic basis of resistance in these strains. As in *S. aureus*, the majority of fusidic acid-resistant *S. epidermidis* isolates carried the *fusB* gene.

**Materials and methods**

CoNS strains isolated from patients as an apparent cause of infection, and presumptively identified as *S. epidermidis*, were collected from the Leeds General Infirmary (LGI) (*n* = 30) and from around Europe (*n* = 20). The identity of these isolates was confirmed as *S. epidermidis* in all cases by PCR amplification and DNA sequencing of a 598 bp region of the *rpoB* gene. Fusidic acid susceptibility was determined by agar dilution in Iso-Sensitest agar (Oxoid, Basingstoke, UK) using inocula in Iso-Sensitest broth (Oxoid) of 10⁶ cfu per spot.

The *fusA* gene was PCR-amplified using oligonucleotide primers *rpsU* and *tufL*² and sequenced with these and three additional primers (AintS1, 5'-TAAGGGTCAGTCAACTTT; AintS2, 5'-TT CAAAAAAGGTGTTC; AintS3, 5'-ATGTATTCACGGAGGA AC). The multiplex PCR assay for *fusB* and *fusC* used oligonucleotide primers *BF* (5'-CTATAATGATATTAATGAGATTTTTGG), *BR* (5'-TTTTTACATATTGACCATCCGAATTGG), *CF* (5'-TTAAAGAAAAAGATATTGATATCTCGG) and *CR* (5'-TTTACAGAATCCTTTTACTTTATTTGG) to generate amplicons of 431 and 332 bp from the *fusB* and *fusC* genes, respectively. The cycling conditions comprised an initial denaturation step (94°C for 3 min), followed by 25 cycles of 94°C (30 s), 57°C (30 s) and 72°C (45 s). The results of the multiplex PCR were confirmed by Southern hybridization.² Genetic typing was performed by DNA sequencing of the repeat region of the gene for accumulation-associated protein (*aap*),⁹ using NCTC 11047 to assign alleles.

**Results and discussion**

All 50 isolates were confirmed as *S. epidermidis* by *rpoB* sequencing. On the basis of the suggested EUCAST fusidic acid resistance breakpoint for *S. aureus* (≥1 mg/L), 23 of 50 (46%) isolates were considered to be resistant to fusidic acid. This figure is substantially higher than that observed in *S. aureus*, as a previously published study described a mean fusidic acid resistance rate of only ~5% in 4065 consecutive *S. aureus* isolates recovered from patients from 20 hospitals in 19 countries.¹⁰ This suggests that *S. epidermidis* has been subjected to strong fusidic acid selection pressure, probably as a result of topical application of the drug to the skin.

The genetic basis for resistance to fusidic acid in these isolates was examined. In previous studies involving the detection of fusidic acid resistance determinants in staphylococci, we employed Southern hybridization.² To simplify the detection of fusidic acid resistance genes, we developed a novel multiplex PCR assay capable of detecting both the *fusB* and *fusC* genes and used the present study with *S. epidermidis* as an opportunity to evaluate this approach. The multiplex PCR assay was initially assessed using DNA from a series of positive and negative control strains, including a sample of *fusB*₆DNA spiked with *fusC*₇DNA which showed that simultaneous detection of both determinants in a single sample was possible (Figure 1).

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**Figure 1.** Agarose gel showing representative results of the multiplex PCR assay for *fusB* and *fusC*. FA, fusidic acid.
Subsequently, the multiplex PCR assay was run in tandem with Southern hybridization on DNA from all 23 fusidic acid-resistant \textit{S. epidermidis} isolates. Results from both approaches were in complete agreement, providing reassurance of the utility of the multiplex PCR method for simultaneous detection of \textit{fusB} and \textit{fusC}.

The \textit{fusB} gene was detected in 18 of the 23 resistant isolates, and two further isolates were found to carry \textit{fusC} (Table 1). As in \textit{S. aureus},\textsuperscript{2} these determinants conferred resistance to concentrations of fusidic acid ranging from 4 to 16 mg/L in \textit{S. epidermidis}. The three isolates exhibiting high-level resistance to fusidic acid (\(\geq 256\) mg/L), and carrying neither \textit{fusB} nor \textit{fusC}, were found to possess a \textit{fusA} mutation encoding the substitution L461K in EF-G when compared with a fusidic acid-susceptible strain (NCTC 11047). This is the same substitution previously reported in fusidic acid-resistant clinical strains of \textit{S. aureus}\textsuperscript{11}

Because 20 of 23 (87\%) fusidic acid-resistant \textit{S. epidermidis} isolates contained a horizontally acquired fusidic acid resistance determinant, which is also functional in \textit{S. aureus}, and given the substantially greater prevalence of resistance in the former, it is reasonable to speculate that \textit{S. epidermidis} may represent a source of fusidic acid resistance determinants for \textit{S. aureus}.

To determine whether the apparent high prevalence of fusidic acid resistance detected in this study was due to the spread of closely related resistant strains, they were subjected to molecular typing via DNA sequencing of the gene-encoding accumulation-associated protein (\textit{aap}).\textsuperscript{9} Using this approach, at least 12 distinct genotypes [B to L (arbitrary letter codes) and \textit{aap}-negative, Table 1] were identified in this collection of 23 isolates. However, as 4 \textit{aap} types (H, K, L and NT; Table 1) included strains that possess different fusidic acid resistance genotypes, this collection appears to comprise at least 16 independent strains, suggesting that fusidic acid resistance has been acquired by multiple genetic lineages of \textit{S. epidermidis}.

In conclusion, the prevalence of resistance to fusidic acid in \textit{S. epidermidis} is apparently much greater than that seen in \textit{S. aureus}, although formal surveillance data will be required to confirm this. Resistance to this antibiotic in \textit{S. epidermidis} is frequently the result of carriage of the \textit{fusB} determinant, reflecting a situation similar to that found in \textit{S. aureus}.

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